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AD-A208 231

# REPORT DOCUMENTATION PAGE

1b. RESTRICTIVE MARKINGS

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4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

| 6a. NAME OF PERFORMING ORGANIZATION | 6b. OFFICE SYMBOL (If applicable) | 7a. NAME OF MONITORING ORGANIZATION |
|---|---|---|
| Naval Ocean Systems Center | NOSC | |

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|---|---|
| San Diego, CA 92152–5000 | |

| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION | 8b. OFFICE SYMBOL (If applicable) | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER |
|---|---|---|
| Civil Engineering Lab | | |

| 8c. ADDRESS (City, State and ZIP Code) | 10. SOURCE OF FUNDING NUMBERS | | | |
|---|---|---|---|---|
| | PROGRAM ELEMENT NO. | PROJECT NO. | TASK NO. | AGENCY ACCESSION NO. |
| Construction Battalion Center<br>Port Hueneme, CA 93043 | 0602233N | MEO2 | RM 33E<br>80 | DN 888<br>573 |

11. TITLE (include Security Classification)

EXPRESSION OF HEAT SHOCK PROTEINS AND METALLOTHIONEIN IN MUSSELS EXPOSED TO HEAT STRESS AND METAL ION CHALLENGE

12. PERSONAL AUTHOR(S)

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| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| professional paper | FROM TO | May 1989 | |

16. SUPPLEMENTARY NOTATION

| 17. COSATI CODES | | | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) |
|---|---|---|---|
| FIELD | GROUP | SUB-GROUP | CIVAPP: Environmental Programs<br>CIVAPP: Marine Biology<br>CIVAPP: Analytical Chemistry |
| | | | |
| | | | |

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Heat stress and metal ion challenge are just two of the numerous stressors capable of inducing the expression of a class of stress proteins commonly known as heat shock proteins. Expression of heat shock proteins (hsps) in response to a variety of stressors appears to be biologically universal. Induction generally coincides with the suppression of "normal" cellular protein synthesis activity, and imparts stress resistance or tolerance to the cell. The mechanism by which the proteins convey tolerance is not clearly understood. Trace metal challenge can also induce the synthesis of metallothionein (MT). MT has been found in the tissues of metal stressed mussels. In this study heat expression of hsps and trace metal induction of hsps and MT was investigated in tissues excised from Mytilus edulis. Protein expression in hemocytes and gill tissue was followed using 35[S]–labeled cysteine or methionine followed by resolution of labeled proteins by sodium dodecyl sulfate polyacrylaminade gel electrophoresis (SDS–PAGE) and autoradiography. Proteins corresponding in molecular weight to hsps were expressed in tissues incubated at 25 and 30°C. Cu (50 µg/liter) was a strong inducer of hsps while MT induction was negligible. Higher Cu concentrations (150 µg/liter) caused a complete inhibition of label incorporation. Cd appeared to be a poor inducer of hsps. MT was induced after a 24–h incubation in 600 µg/liter Cd. No increase in MT levels was observed as a result of heat stress.

Published in *Marine Environmental Research* 24 (1988).

| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT | 21. ABSTRACT SECURITY CLASSIFICATION |
|---|---|
| ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS | UNCLASSIFIED |

| 22a. NAME OF RESPONSIBLE PERSON | 22b. TELEPHONE (include Area Code) | 22c. OFFICE SYMBOL |
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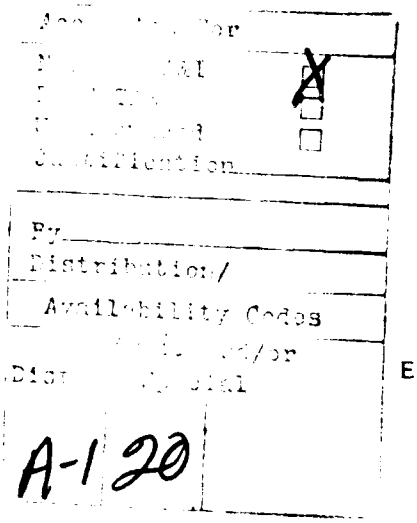
*Marine Environmental Research* 24 (1988) 211–214

# Expression of Heat Shock Proteins and Metallothionein in Mussels Exposed to Heat Stress and Metal Ion Challenge

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*Heat stress and metal ion challenge are just two of the numerous stressors capable of inducing the expression of a class of stress proteins commonly known as heat shock proteins. Expression of heat shock proteins (hsps) in response to a variety of stressors appears to be biologically universal. Induction generally coincides with the suppression of 'normal' cellular protein synthesis activity, and imparts stress resistance or tolerance to the cell. The mechanism by which the proteins convey tolerance is not clearly understood. Trace metal challenge can also induce the synthesis of metallothionein (MT). MT has been found in the tissues of metal stressed mussels. In this study heat expression of hsps and trace metal induction of hsps and MT was investigated in tissues excised from* Mytilus edulis. *Protein expression in hemocytes and gill tissue was followed using 35[S]-labeled cysteine or methionine followed by resolution of labeled proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Proteins corresponding in molecular weight to hsps were expressed in tissues incubated at 25 and 30°C. Cu (50 μg/liter) was a strong inducer of hsps while MT induction was negligible. Higher Cu concentrations (150 μg/liter) caused a complete inhibition of label incorporation. Cd appeared to be a poor inducer of hsps. MT was induced after a 24-h incubation in 600 μg/liter Cd. No increase in MT levels was observed as a result of heat stress.*



*Marine Environ. Res.* 0141-1136/88/$03·50 

Induction of hsps by heat stress has been demonstrated in prokaryotes and eukaryotes (see review in ref. 1), and is thought to be a universal biological response. The expression of these proteins is characteristically linked with a reduction in the synthesis of cellular proteins present in the cell prior to stress. Many stressors other than heat, for example transition metals, oxidizing agents, sulfhydryl reagents and viruses, have been shown to induce the heat shock response.[2] In many instances hsp induction has been shown to be coincidental with the attainment of stress tolerance.[3] Therefore, the conveyance of stress tolerance is the suspected function of these proteins. Multiple proteins are induced, but the character and number involved varies depending on the organism or stress. Because of this diversity the actual underlying mechanisms of the response are not well understood.

We were interested in the potential utility of hsp induction as a general stress indicator and examined the heat shock response of mussels. In addition, it is well known that certain trace metals induce the expression of proteins other than hsps, and in particular the induction of metallothioneins.[4] MT has been a focal point of trace metal toxicity studies, and it was therefore of interest to determine whether hsps as well as MT were induced, over a short-term exposure, by Cd and Cu.

Mussels, *Mytilus edulis*, were collected from San Diego Bay, California. Ambient seawater temperature at the collection site was 15°C. Fifteen minutes after collection the mussels were placed in static seawater aquaria kept at 15°C. The mussels were acclimated for 9 days; water was exchanged daily with fresh bay water. Following acclimation the mussels were incubated at different temperatures (15, 20, 25 and 30°C) or with Cd (200 and 600 $\mu$g/liter) or Cu (50 and 150 $\mu$g/liter) in 0·45$\mu$ filtered seawater (salinity 34‰, pH 7·9–8·1). Controls were held at 15°C. Whole animals were incubated under control or test conditions for 4 or 28 h; hemocytes and gill tissue were then removed and pooled from six individuals, incubated *in vitro* under the same conditions in 100 $\mu$l 0·45 $\mu$ filtered seawater and 25 $\mu$Ci/ml of either 35[S]-methionine or 35[S]-cysteine (Amersham Corp., >600 Ci/mmol) for 20 h. Afterwards, the samples were centrifuged at 1500 × *g* at room temperature for 15 min, the supernatant discarded, pellets washed twice in 0·45 $\mu$ filtered seawater, resuspended in 100 $\mu$l distilled water and disrupted by sonication, recentrifuged at 10 000 × *g* at 15°C for 15 min and the supernatant transferred to a clean microcentrifuge tube and the pellet discarded. The concentration of DNA in the samples was utilized as an analog of the total cell number. A 10 $\mu$l aliquot from each sample was used for DNA determinations using the 4,6-diamidino-2-phenylindole (DAPI) method of Hukkelhoven *et al.*[5] Samples were diluted to normalize for DNA concentration and then mixed 1:1 with SDS-PAGE 'loading' buffer, 0·13M Tris, 0·14M SDS, 29 $\mu$M bromophenol blue, 10% 2-mercaptoethanol and 20% glycerol, and placed in boiling water for 2 min.

Samples from cells incubated with 35[S]-methionine were separated on 10% SDS-PAG for resolution of hsps and 35[S]-cysteine incubated samples on 15% SDS-PAG for MT, using the discontinuous buffer system of Laemmli.[6] The gels were stained in 50% ethanol, 5% glacial acetic acid, 0·1% Coomassie brilliant blue (R-250) for 90 min and destained in a Biorad destain chamber for 90 min, then dried in a vacuum slab dryer for 2 h. Dried gels were sandwiched in a film cassette with Kodak XAR X-ray film for 12 h to visualize proteins with incorporated label. Stained gels and autoradiographs were scanned with a laser densitometer.

Prominent hsps of 70, 51 and 28 kDa were identified in both gill and hemocytes after 24 h at 25 and 30°C. A general decline in overall protein synthesis was also indicated. Comparison of control and test lanes by densitometry showed the total integrated area of test lanes to be half to one-third that of controls.

Autoradiographs of proteins from gill and hemocytes exposed to 50 $\mu$g/liter Cu for 24 h showed nearly identical patterns of induced methionine label incorporation. Tissues exposed to 150 $\mu$g/liter Cu showed a complete inhibition of label incorporation. We have not ascertained whether this was a result of translational inhibition or some other mechanism such as the inhibition of cellular uptake of the label. However, Thiele *et al.*[7] reported the total inhibition of protein synthesis in yeast by Cu, though at the very high concentration of 19 mg/liter.

Cd, on the other hand, only induced hsps after 48 h at the 600 $\mu$g/liter concentration. Heikkila *et al.*[8] reported a transient Cd hsp response in Chinook salmon embryo cell line CHSE-214. Hsp levels were maximal at 30 to 60 min and returned to control levels after 3–4 h when exposed to 550 $\mu$g/liter Cd. However, in our own work we have generally found mussels to be far more sensitive to Cu than Cd, so these results seem to be consistent with previously gathered toxicity data (not published).

MT induction, as shown by 35[S]-cysteine incorporation at the position on a gel where mussel MT is normally resolved, at an approximate MW of 10 000 (unpublished data from this laboratory), was not apparent above control levels in heat-stressed or Cu-stressed cells. Induction by Cd was observed after 24 h at 600 $\mu$g/liter in hemocytes.

The data suggest that heat shock is a general response to acute stress, and that the induction of heat shock in mussels can be determined non-destructively by examining serum hemocytes. It is also interesting to note that the hemocytes clearly demonstrated MT induction. In the future it will be interesting to examine the possible development of tolerance in heat shock responders. We also hope to develop a more well-defined correlation between cellular MT concentration and 35[S]-cysteine incorporation into MT. This will provide a means of measuring synthesis levels in cells from freshly collected mussels representing different field populations.

## REFERENCES

1. Lindquist, S. *Ann. Rev. Biochem.*, **55**, 1151–91 (1986).
2. Nover, L. In *Heat Shock Response of Eukaryotic Cells* (L. Nover, ed.), Springer-Verlag, Berlin, pp. 8–9, 1984.
3. Li, G. C., Shrieve, D. C. & Werb, Z. In *Heat Shock from Bacteria to Man* (M. J. Schlesinger, M. Ashburner & A. Tissieres, eds), Cold Spring Harbor Laboratory, New York, pp. 395–404, 1982.
4. Hamer, D. H. *Ann. Rev. Biochem.*, **55**, 913–51 (1986).
5. Hukkelhoven, M. W. A. C., Vromans, E., Markslag, A. M. G. & Vermorken, A. J. M. *Anticancer Res.*, **1**, 341–4 (1981).
6. Laemmli, U. K. *Nature*, **227**, 680–5 (1970).
7. Thiele, D. J., Walling, M. J. & Hamer, D. H. *Science*, **231**, 854–6 (1986).
8. Heikkila, J. J., Schultz, G. A., Iatrou, K. & Gedamu, L. *J. Biol. Chem.*, **257**, 12000–5 (1982).

G V-Pickwell Rec'd 6-07-88

# Marine Environmental Research

VOL. 24 1988

*SPECIAL ISSUE*

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